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Autoclaving Eliminates Hepatitis C Virus From a Hemodialysis Monitor Contaminated Artificially

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Nosocomial transmission of the hepatitis C virus (HCV) has become the principal cause of HCV infection in hemodialysis units. Because HCV particles may pass through dialysis membranes and backfiltration occurs with high performance membranes, HCV transmission from contaminated dialysis monitors is likely. Thus it is important to have effective measures to disinfect hemodialysis monitors. In this study, autoclaving dialysate circuits were examined to establish an effective method to eliminate HCV particles from a monitor contaminated artificially. The dialysis monitor was contaminated in 2 different experiments with a 1/10 and 1/5 dilution of a serum pool containing $1.2 \pm 0.3 \times 10^6$ HCV genome copies/ml. During perfusion 2 samples were taken from the drainage tube at 5 and 10 minutes. After perfusion, the dialysate circuit was autoclaved at 120°C for 20 minutes. Four samples were then taken from the autoclaved circuits and another from the drainage, which had not been autoclaved. The viral titer in the samples from the drainage before sterilization was similar to that of the serum dilution, showing the homogeneous distribution of the serum dilution in the dialysis circuits. After autoclaving, HCV RNA was not detectable in the samples obtained from the autoclaved circuits, whereas it was positive in the sample from the drainage. These results show that autoclaving is an effective method to eliminate HCV particles from contaminated hemodialysis monitors. ***J. Med. Virol. 60:139–143, 2000.***

KEY WORDS: hemodialysis; hepatitis C virus; autoclaving; dialysis circuit

INTRODUCTION

Patients treated by chronic hemodialysis are at high risk of hepatitis C virus (HCV) infection [Zeldis et al., 1990; Bukh et al., 1993; Barril and Traver, 1995]. Although the decrease in the number of blood transfusions undertaken in dialysis patients, due to the use of erythropoietin and anti-HCV screening in blood banks, has reduced the incidence of transfusion-associated HCV infection in the hemodialysis setting, a relatively high incidence still exists of new HCV infections in hemodialysis units. This fact illustrates the increasing importance of nosocomial HCV transmission in chronic hemodialysis [Allander et al., 1994; Sampietro et al., 1995]. In this sense, cross-contamination from HCV-infected hemodialysis patients has been demonstrated [Sampietro et al., 1995; Le Pogam et al., 1998]. Whether this transmission occurs by direct patient-to-patient contact or by HCV-contaminated dialysis monitors is still a matter of debate. While some researchers have attributed the spread of HCV among hemodialysis patients to the use of HCV-infected monitors [Seme et al., 1997; Le Pogam et al., 1998], others state that this type of HCV transmission is negligible [Jadoul et al., 1998]. It has been shown, however, that HCV particles may be adsorbed and cross through dialysis membranes [Hayashi et al., 1997; Valtuille et al., 1998]. This finding and the fact that backfiltration may occur, especially when high performance membranes are used [Caramelo et al., 1994], make HCV transmission from contaminated dialysis monitors likely. Based on these data, the existence of effective methods to disinfect hemodialysis monitors is important.

The infection process between 2 hemodialysis sessions is usually performed by chemical means, although no tests have been performed to discover whether these chemical agents destroy HCV. A new dialysis monitor that sterilizes the dialysate circuit by autoclaving has been developed recently, so we evaluated sterilization of a dialysis monitor by autoclaving as a method to eliminate HCV particles from a monitor contaminated artificially.

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MATERIALS AND METHODS

A pool of 900 ml of serum was obtained from therapeutic phlebotomies (500 ml of blood in each phlebotomy) performed in 3 patients with elevated serum ferritin levels (>200 ng/ml) and histologically proven chronic hepatitis C. Histologic findings also demonstrated liver siderosis in the 3 patients. All patients were anti-HCV positive and had high HCV RNA levels in serum. None had hepatitis B surface antigen (HBsAg), anti-hepatitis D antibodies (anti-HDV), or anti-human immunodeficiency virus antibodies (anti-HIV). Other causes of liver disease (autoimmune hepatitis, alcoholic hepatitis) were also discarded.

HCV RNA Detection and Quantification

HCV RNA was detected in serum and in the samples obtained from the dialysis monitor by reverse transcription-polymerase chain reaction (RT-PCR) using primers of the highly conserved 5′-non-coding (NC) region of the HCV genome, as previously described. HCV RNA quantification was performed with the Amplicor HCV Monitor kit (Roche Diagnostic Systems, Branchburg, NJ) according to the manufacturer's instructions.

Those samples negative for HCV RNA by quantitative and qualitative RT-PCR were ultracentrifuged to increase assay sensitivity. Samples were clarified by centrifugation at 12,000*g* for 10 minutes. The supernatant (1 ml) was layered onto a sucrose cushion consisting of 2 ml of 1 g/ml sucrose in TEN buffer (50 mM Tris-HCl, pH 8.0, 1 mM EDTA, and 150 mM NaCl) and centrifuged at 50,000*g* for 24 hours at 10°C in a Beckman SW60 Ti rotor (Beckman Co., Palo Alto, CA). The pellet was resuspended in 250 μl of sterile distilled water and, after the total RNA was isolated with the Trizol LS Reagent (Gibco BRL, Gaithersburg, MD), RT-PCR was carried out using 5′-NC HCV region primers, as described above. All PCRs were undertaken at least in triplicate by 2 different workers. The same results were obtained in all cases.

Artificial Contamination of the Dialysis Monitor

The dialysis monitor used in this study was a Miro-Clav C (Baxter S.A., Spain), which sterilizes the dialysate circuit by autoclaving at 120°C for 20 minutes.

To artificially contaminate the dialysis monitor, the serum pool was diluted 1/10 (final volume 500 ml) in normal saline solution. A 3 ml aliquot was taken to determine the viral titer. The sample was passed through the monitor at a flow rate of 50 ml/min and ultrafiltration was maintained at 3 l/hr. We attempted to reproduce the in vivo standard procedure conditions. A T tube was inserted between the 2 dialysate connectors (Fig. 1). The diluted HCV sample was infused throughout this T tube. The monitor temperature was maintained at 37°C. During perfusion, 2 samples were taken from the drainage at 5 and 10 minutes after starting. As a dialysate, distilled water was used rather than a hemodialysis concentrate to avoid interference from divalent cations in the PCRs. The monitor

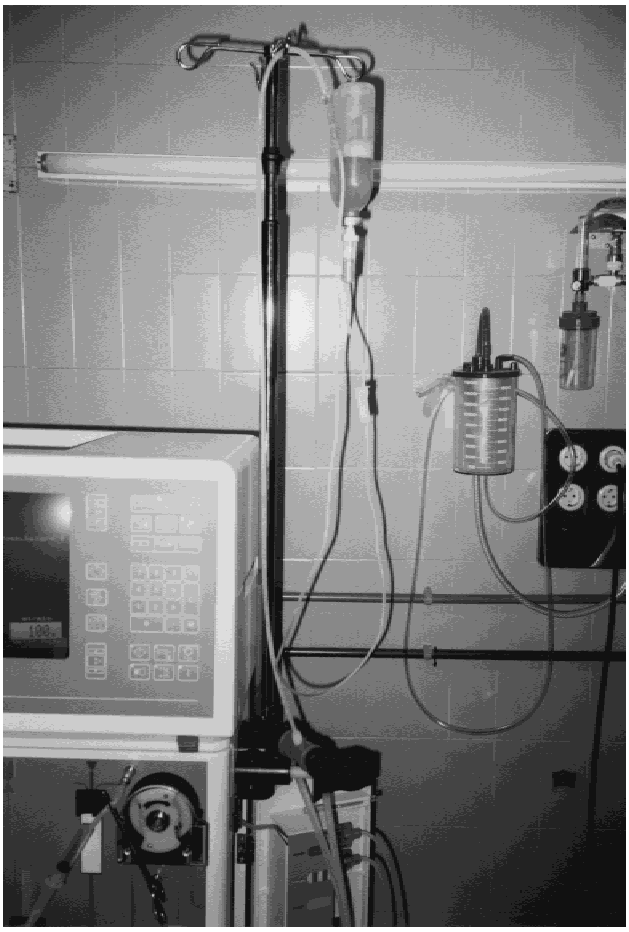

Fig. 1. Photograph of the dispositive used to pass the diluted serum pool through the dialysis monitor.

was shut off for 30 minutes after perfusion. Citric acid was then passed through the dialysate circuit for 5 minutes and autoclaved for 20 minutes at 120°C. Finally, the monitor was shut off and permitted to cool to room temperature for 150 minutes. At that time, 4 samples (3 ml each) were taken from the internal circuits of the machine from the points shown in Figure 2. An additional sample was taken from the drainage after autoclaving.

The experiment was repeated with the serum pool diluted 1/5 (final volume 250 ml). The rest of the procedure and the sample collection were similar to that described above.

RESULTS

The HCV titer of the serum pool was $1.2 \pm 0.3 \times 10^6$ genome copies/ml. The initial 1/10 dilution of the serum pool in saline buffer had a HCV RNA concentration of $3.3 \pm 0.4 \times 10^5$ genome copies/ml, whereas the viral titer of the samples taken from the drainage at 5 and 10 minutes during perfusion was $3.4 \pm 0.2 \times 10^5$ and $3.2 \pm 0.4 \times 10^5$ genome copies/ml, respectively. This result shows that the serum pool dilution distribution in the internal circuits of the monitor was homogeneous. On

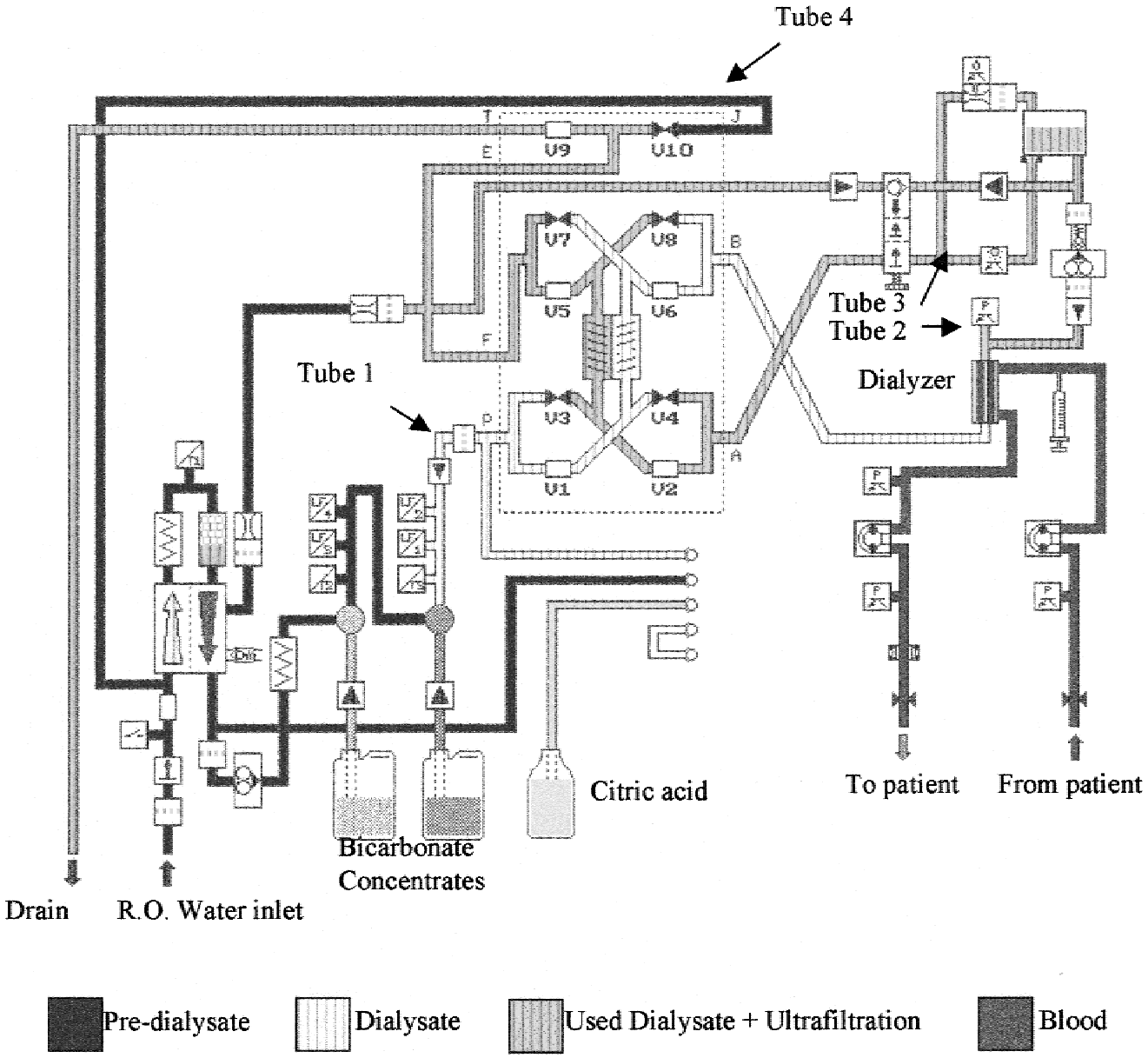

Fig. 2. Schematic representation of the internal circuits of the dialysis monitor used in this study. Points from where the samples were taken after autoclaving are shown by arrows.

the other hand, the samples taken from the internal circuits of the monitor after sterilization by autoclaving were negative for HCV RNA. On the contrary, the sample obtained from the drainage (which is outside the part of the monitor which is autoclaved) after sterilization was HCV RNA positive with a titer of $1.0 \pm 0.3 \times 10^4$ genome copies/ml.

Similar results were obtained when a 1/5 dilution of the serum pool was used to contaminate the monitor. In this case, the viral titer of the dilution was $1.0 \pm 0.6 \times 10^6$ genome copies/ml, whereas the viral concentrations in the eluates obtained from the drainage at 3 and 5 minutes during perfusion were $5.6 \pm 0.6 \times 10^5$ and $7.2 \pm 1.2 \times 10^5$ genome copies/ml, respectively.

Once again, the samples taken from the internal circuits of the monitor after sterilization were HCV RNA negative. The HCV RNA concentration of the sample obtained from the drainage after autoclaving was $1.1 \pm 0.7 \times 10^4$ genome copies/ml. To confirm these results, all samples were tested again for the presence of HCV RNA by a qualitative RT-PCR. After performing this analysis, HCV RNA was not detectable in the samples that were HCV RNA negative by Amplicor, whereas samples that were positive by the quantitative PCR assay were also positive by the qualitative RT-PCR.

Finally, to confirm further that the lack of HCV RNA detection in the samples from the internal circuits after sterilization was not due to the presence of viral RNA at concentrations under the detection limits of the PCR technique used, negative samples were ultracentri-

fuged and tested for HCV RNA again by RT-PCR. HCV RNA was not detectable after the ultracentrifugation in all cases.

DISCUSSION

Several reports have shown that nosocomial transmission is becoming the main cause of HCV infection in hemodialysis units [Allander et al., 1994; Sampietro et al., 1995; Jadoul et al., 1998; Le Pogam et al., 1998]. Nevertheless, it has not been established fully whether the spread of HCV among hemodialysis patients occurs through direct patient-to-patient contact or through HCV-infected monitors. Data that HCV can pass through or adhere to HCV dialysis membranes and the possibility of backfiltration suggest that at least a small percentage of HCV infection in hemodialysis units may be due to contaminated monitors. To avoid this problem, some hemodialysis units have adopted the policy of using separate rooms or separate monitors to dialyze anti-HCV positive patients [Pol et al., 1993; Al-Meshari et al., 1995; Traver and Barril, 1996]. However, this option is not completely efficient for several reasons. There are dialysis patients with HCV RNA in serum who are infectious even though no anti-HCV antibodies have been detected for a long period of time [Caramelo et al., 1996]. Furthermore, in cases of acute HCV infection there is a "window period," which may last from 4 weeks to 1 year, during which the patient has circulating viral RNA in the absence of anti-HCV antibodies [Miyamura et al., 1990]. In addition, antibodies against 1 HCV genotype do not protect against infection by a different genotype [Farci et al., 1992]. The use of the same monitor for patients infected with different HCV genotypes may lead to superinfection of a given patient with a distinct HCV genotype. Finally, this measure cannot be applied in cases of acute hemodialysis.

It has been reported recently that strict adherence to the universal precautions proposed by the Centers for Disease Control (Atlanta, GA) may prevent nosocomial transmission of HCV [Jadoul et al., 1998]. It is difficult to assure the correct application of these procedures, however, particularly in units with a large number of hemodialysis patients and especially in those with high HCV prevalence. Furthermore, although adherence to the universal precaution measures may prevent direct patient-to-patient infection or infection of patients by health care workers and vice versa, it cannot avoid the contamination of dialysis monitors through the dialysis membranes, and consequent infection by backfiltration.

These facts indicate that, even if it is assumed that infection through contaminated monitors accounts for a small percentage of nosocomial HCV transmission, efficient methods to disinfect dialysis monitors are needed. A new hemodialysis monitor that sterilizes the dialysate circuits by autoclaving has been developed recently. Previous studies have demonstrated that this sterilization procedure more efficiently eliminates the biofilm than the conventional chemical procedures used to disinfect these hemodialysis monitors [Degremont et al., 1998]. Our aim was not to compare one method with another, but to determine whether or not sterilization by autoclaving effectively eliminates HCV contamination.

The results show unequivocally that vapor sterilization at 120°C for 20 minutes is sufficient to eradicate HCV contamination of the monitor. It may be argued that the HCV was washed out from the internal monitor circuits by the citric acid passed through the monitor before autoclaving. The fact that HCV was detectable in the drainage, washed out by the acid but not autoclaved, makes this hypothesis unlikely.

Finally, it should be noted that the number of HCV particles contaminating the monitor in the present report was much higher than that reported in cases of HCV contamination from hemodialysis patients [Valtuille et al., 1998], and even under these unfavorable conditions, vapor sterilization was a very effective method for the elimination of HCV contamination.

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